

AD-A079 298

ILLINOIS BENEDICTINE COLL LISLE  
SYNTHESIS OF PURINE NUCLEOSIDE AND NUCLEOTIDE ANALOGS AS ANTIPA--ETC(U)  
SEP 79 J P NEENAN  
DAMD17-78-C-8058

F/G 6/15

JNCLASSIFIED

4

NL

1-1  
AC  
107-108



END

DATE

FORMED

2 80

100

LEVEL

REPORT NO. 4 ✓

SYNTHESIS OF PURINE NUCLEOSIDE  
AND  
NUCLEOTIDE ANALOGS AS ANTIPARASITIC AGENTS

ANNUAL REPORT 1 AND FINAL REPORT

John P. Neenan, Ph.D.  
1 September 1979

Supported by  
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-78-C-8058

Illinois Benedictine College ✓  
Lisle, Illinois 60532

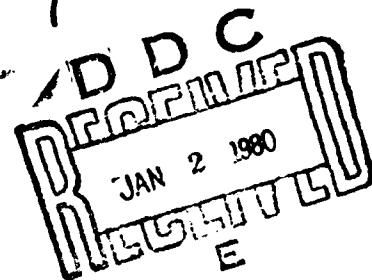
Approved for public release; distribution unlimited

The findings in this report are not to be construed as an  
official Department of the Army position unless so designated by  
other authorized documents.

79 12 31 015

ADA 079298

DDC FILE COPY



S/L  
394205

11/6

## SUMMARY

The purpose of the research was to conduct studies on the synthesis of purine nucleoside and nucleotide analogs as anti-parasitic agents. The primary target compounds were 5'-deoxytubercidin 5'-amino-5'-deoxytubercidin (nucleoside analogs) and 5'-fluoro-5'-deoxytubercidin (a nucleotide analog.)

One target, 5'-deoxytubercidin, was successfully prepared in an efficient two-step procedure and submitted for screening.

Several attempts to prepare the 5'-amino- and 5'-fluoro-derivatives of tubercidin either by direct routes or via blocked tubercidin were unsuccessful. Due to problems encountered in these attempted syntheses, work on 5'-derivatives of two other purine nucleoside antibiotics - puromycin aminonucleoside and 6-methylmercaptapurine riboside - was not initiated. However, work on 5'-derivatives of nebularine was performed and some success was achieved in this series.

Nebularine was synthesized by a literature method and submitted for screening. A two-step synthesis of 5'-deoxynebularine from nebularine via 5'-deoxy-5'-iodonebularine was accomplished even though an analytical sample is not yet in hand.

Additional 5'-deoxytubercidin and its synthetic precursor, 5'-chloro-5'-deoxytubercidin were prepared when attempts to synthesize the 5'-amino- and 5'-fluoro- derivatives had to be abandoned because the contract was close to termination. Unfortunately, these samples are not analytically pure as of this report.

In summation, 1.6g of nebularine and over 600 mg of 5'-deoxytubercidin were submitted for screening during the project. Additional 5'-deoxytubercidin as well as samples of 5'-chloro-5'-deoxytubercidin and 5'-deoxynebularine are in hand but require further purification.

Accession For	
HTIS GRAM	<input checked="checked" type="checkbox"/>
DMS TAB	<input type="checkbox"/>
Unsubstantiated	<input type="checkbox"/>
Justification	
By	
Distribution	
Availability Codes	
Dist	Special

## FOREWORD

This research was conducted under contract No. DAMD17-78-C-8058 during the period 1 September 1978 to 31 August 1979. Dr. John P. Neenan served as Principal Investigator. Four undergraduate students conducted the research under Dr. Neenan's supervision: Stephen Andracki, Steve Howard, Peter Lodestro, and Walter Vogt. Stephen Andracki and Steve Howard worked on the project through its entire period, and the accomplishments of the project are largely attributable to their superlative effort. Peter Lodestro served as replacement for Walter Vogt, who had to leave the project because of a heavy course load. Peter filled an important logistic niche as supplier of nebularine via catalytic reduction of 6-chloropurine riboside.

Grateful acknowledgements are also due to Drs. Pete Johnson at Illinois Institute of Technology, Malcolm MacCross at Argonne National Laboratory and Henry Winkler at Illinois Benedictine College for their assistance with NMR spectra of the target compounds.

## TABLE OF CONTENTS

	<u>Page</u>
Summary	2
Foreword	3
Technical Presentation	
Background	5
Chemistry	5 - 9
Recommendations	10
Experimental Section	10 - 13
Literature Cited	14

## TECHNICAL PRESENTATION

### BACKGROUND

A number of purine nucleoside antibiotics such as puromycin aminonucleoside and tubercidin are known antiparasitic agents.<sup>1-4</sup> Jaffe has proposed that the susceptibility of pathogenic helminths and protozoa to fraudulent purine, in contrast to pyrimidine, nucleosides probably results from a deficiency in enzymes that synthesize the purine ring *de novo*. Unfortunately these antibiotics can become incorporated into mammalian nucleic acids and hence are toxic to host cells.

The purpose of this research effort was to modify the parent nucleosides such as tubercidin at 5'-carbon by removal of the hydroxyl group, which is phosphorylated by kinases to provide nucleoside 5'-triphosphates for DNA and RNA synthesis. The hydroxyl group would be replaced by hydrogen, by amino to afford nucleoside analogs,<sup>5,6</sup> or fluorine to yield nucleotide analogs. The resultant 5'-substituted derivatives are thus designed to inhibit nucleoside and nucleotide kinases as well as other parasitic enzymes.

Mammalian cells, on the other hand, could conceivably override such metabolic blockades, especially those imposed on salvage nucleoside kinases, because host cells can synthesize purine nucleotides *de novo* and are not restricted to salvage synthesis pathways. Here then is a means for achievement of selective toxicity against parasites.

### CHEMISTRY

Borchardt et al.<sup>8</sup> first converted tubercidin (compound 1, scheme 1) to 5'-chloro-5'-deoxytubercidin (2) in 37% yield by use of the direct halogenation method of Kikugawa and Ichino.<sup>9</sup> By use of this method and by cold temperature isolation procedure, we prepared compound 2 quantitatively. (See the Experimental Section.)

Dechlorination of 2 by the method of Wang and Hogenkamp then afforded target 5'-deoxytubercidin<sup>10</sup> (3). The overall yield of crystalline and chromatographically homogeneous 3 was repeatedly found to be 49%. However, recrystallization was always required to afford analytically pure material.

In our last scaled-up preparation of 3, over 1.5g of 5'-deoxytubercidin was obtained - again in 48% overall yield. Unfortunately a large amount of product was lost when the flask broke during recrystallization. Over half the product was recovered, chromatographed over Dowex [H<sup>+</sup>] ion-exchange resin, and crystallized from reagent alcohol. However, the final analysis of this sample showed that it still required recrystallization. (Calcd. for C<sub>11</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>: C, 52.79; H, 5.64; N, 22.39. Found: C, 52.37; H, 5.67; N, 19.29).

Two analytically pure samples of 5'-deoxytubercidin were previously submitted for screening.

Both 5'-deoxytubercidin and the chloro intermediate 2 were characterized by NMR. Their spectra resembled those of other 5'-deoxynucleosides and 5'-chloro-5'-deoxynucleosides reported by Wang and Hogenkamp.<sup>10</sup> The IR spectra were largely

uninformative except in the case of compound 2 where a C-Cl stretching band at  $610\text{cm}^{-1}$  was observed. This peak was absent in the ir spectrum of 5'-deoxytubercidin (3), which also gave a negative Beilstein flame test in contrast to chloro-derivative 2. The UV spectra of both 2 and 3 were indistinguishable from that of tubercidin.

To ensure the isomeric purities of compounds 2 and 3, small samples of each were converted quantitatively to the corresponding 2,'3'-O-isopropylidene derivative, (4 and 5) which ran with the solvent front on thin-layer chromatograms (Table I). Any 2'-or 3'-substituted tubercidin in the samples would not have formed an isopropylidene derivative.

Compound 2 decomposes in boiling water to form at least two products as observed on tlc. The half-life of 2 under those conditions is less than 2 hours. Presumably, the pathway of decomposition is initial formation of the N<sup>3</sup>,5'-cyclonucleoside-5'-cyclonucleoside<sup>11,12</sup> followed by ring opening of the aglycone. Even at room temperature, 3 gradually decomposes so it is best stored in a freezer. Compound 2 on the other hand is quite stable to prolonged boiling water, and thus can be sterilized and conveniently stored at room temperature.

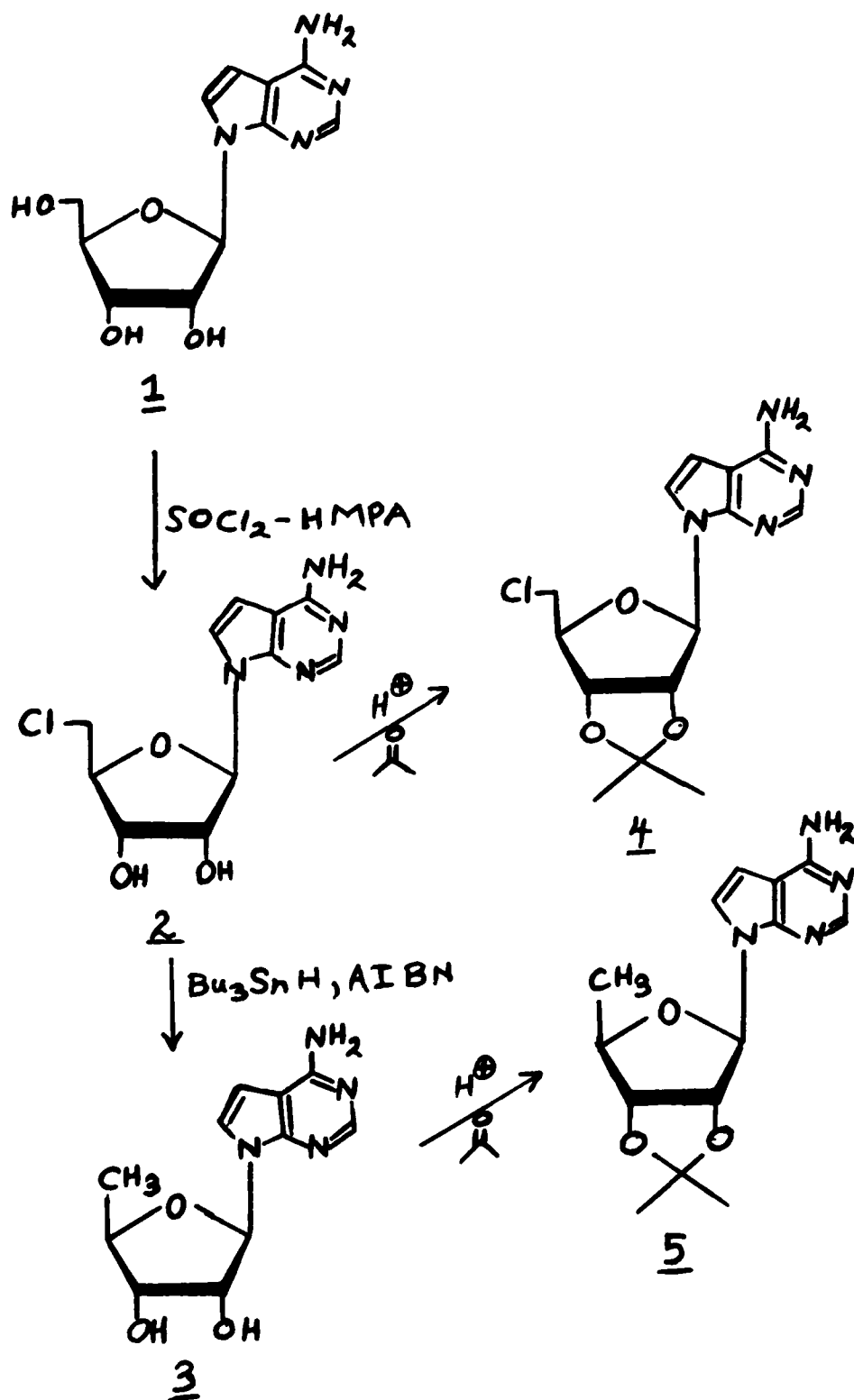
Compound 3 was first prepared in low yield by Anzai and Matsui<sup>12</sup> via a grueling six-step route from tubercidin. These workers had to resort to column chromatography repeatedly for isolation of intermediates from product mixtures. In contrast, the route we report can afford 5'-deoxytubercidin in good yield requires only one ion-exchange chromatography, and is easily reproducible.

Our various unsuccessful attempts to prepare 5'-deoxy-5'-fluorotubercidin and 5'-amino-5'-deoxytubercidin were largely discussed in the quarterly reports. We will summarize these efforts briefly and cover work done since the last report.

Encouraged by the successful synthesis of 5'-chloro-5'-deoxytubercidin with  $\text{SOCl}_2$ -hexamethylphosphoramide (HMPA) reagent,<sup>8,9</sup> we made several attempts to prepare 5'-deoxy-5'-fluorotubercidin analog with  $\text{SOF}_2$ -HMPA either in a bomb or in a three-neck flask. At first we were encouraged because even though much decomposition occurred, one product has an R<sub>x</sub> value identical to compound 2 in several tlc and paper chromatography systems. We expected that the desired 5'-fluoro-derivative would indeed have an R<sub>x</sub> similar to 2. After extensive paper chromatography we isolated this product and found it to be identical to 2 not only by chromatography, but by NMR, by its half-life in boiling H<sub>2</sub>O, and finally by elemental analysis. Both the  $\text{SOF}_2$  we prepared ourselves and that which we purchased contained  $\text{SOCl}_2$  used in synthesis.<sup>13</sup>

Other attempts to prepare the fluoro derivative employed diethyl (2-chloro-1,1,2-trifluoroethyl) amine (CTT)-- a reagent for direct conversion of hydroxyl to fluoro in steroids and cephalosporine.<sup>14</sup> In repeated experiments, we treated tubercidin with freshly prepared CTT in either DMF or HMPA. No product having the expected mobility on tlc of the desired product was found. Indeed both tubercidin and nebularine were largely inert to this reagent.

SCHEME I





Several unsuccessful attempts during the summer were made to prepare 5'-O-p-nitrobenzenesulfonyl tubercidin by the method of Hampton<sup>15</sup> in hopes that subsequent reaction in situ with tetrabutylammonium fluoride or azide into the 5'-carbon-the resultant 5'-azido-5'-deoxytubercidin would have served as synthetic precursor to target 5'-amino-5'-deoxytubercidin. In one experiment with lithium azide we subjected the crude reaction mixture to catalytic hydrogenation and tlc showed that a trace ninhydrin-positive spot had formed which may have been the desired 5'-amino-5'-deoxytubercidin. We could not reproduce this result.

Attempts to prepare either 5'-azido-5'-deoxytubercidin or the corresponding nebularine derivative by a recently reported one-step method<sup>16</sup> also failed.

Attempts to selectively block the 6-amino group with benzoyl or acetyl by the method used on cytidine by Watanabe and Fox<sup>17</sup> lead to incomplete reaction. Thus, when tubercidin was refluxed in methanol in the presence of acetic anhydride most of the tubercidin remained unreacted as monitored by tlc.

The pK of the 6-amino group in tubercidin is higher than that of the 4-amino in cytidine, and presumably protonation inhibits the reaction. Attempts to drive the reaction forward by addition of pyridine as proton scavenger and excess acetic anhydride led to peracetylation as judged by tlc.

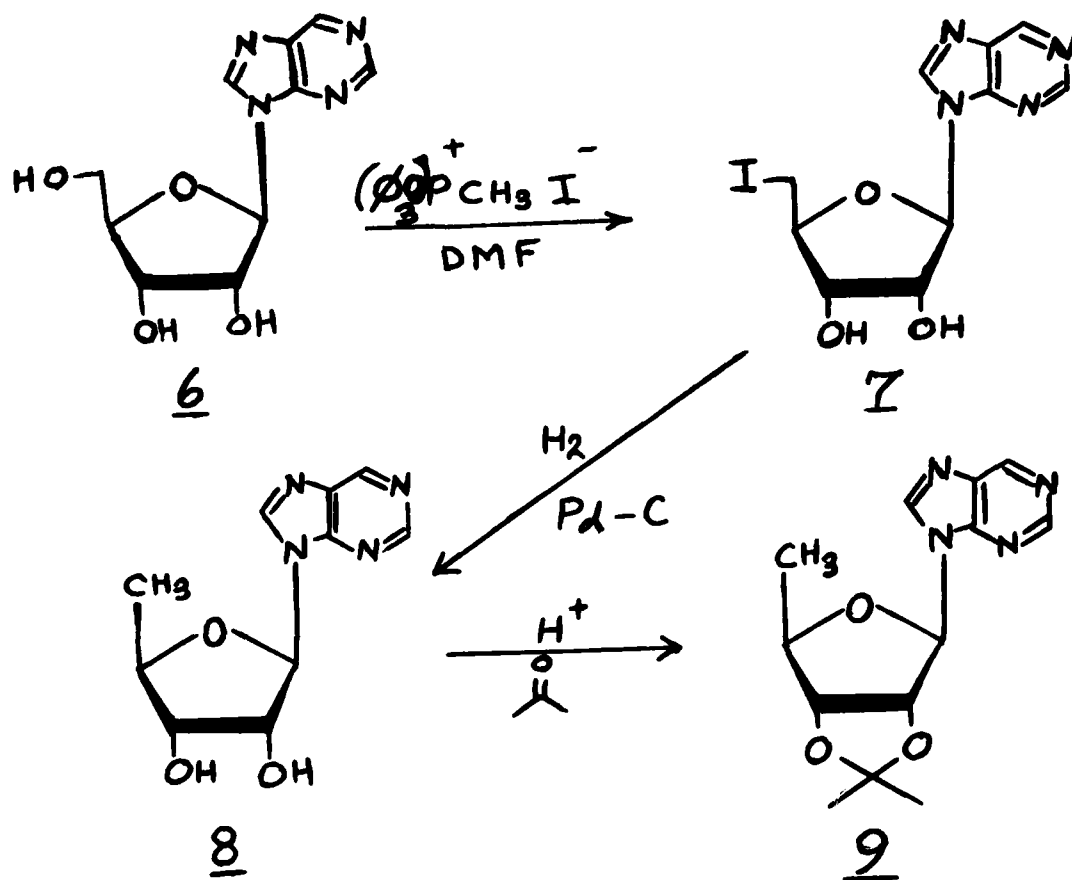
Faced with a severe time constraint we decided to abandon work in the tubercidin series and turn our attention to the nebularine series where we encountered some success.

Treatment of nebularine (6)<sup>18</sup> with methyltriphenoxy-phosphonium iodide<sup>20</sup> in DMF led to complete reaction and formation of a major product which was isolated by extraction, paper chromatography, and finally column chromatography. Due to the extensive work-up procedure and reluctance on the part of the product to crystallize, the yield was low. Nevertheless, we have evidence that the product isolated was indeed 5'-iodo-5'-deoxynebularine (compound I, Scheme II).

The product has the same UV spectrum in acid, base, and water as nebularine itself.<sup>18</sup> As expected of a less polar compound it is homogeneous and moves faster on tlc than nebularine. NMR did not confirm the structure but, on the other hand, was not incompatible with it. An analytically pure sample was not obtained even though attempts were made to crystallize the product from several solvents - even after repeated chromatography. Elemental analysis did, however, confirm the presence of iodine, which was also found by the silver nitrate test.

We decided to convert 7 to 5'-deoxynebularine (8) by catalytic hydrogenation over Pd on carbon in the presence of sodium acetate. The reaction was quantitative. Thus, a single new derivative (8) of nebularine of lower R<sub>f</sub> than 7 was observed on tlc. The product was chromatographed over a silica gel column and attempts were made to crystallize but only sodium acetate was obtained in this manner. The mother liquors were condensed to a brown residue which according to UV contains over 600 mg of target compound 8, the structure of which was assigned by UV, tlc, and quantitative isopropylidenation to 9. Thus, while we do not have a crystalline sample of 5'-deoxynebularine as of this final report, the route to it is apparently feasible. (Scheme II).

SCHEME II



## RECOMMENDATIONS

The Principal Investigator will submit a new contract proposal from the University of Arizona with revised routes to 5'-amino-5'-deoxytubercidin and 5'-fluoro-5'-deoxytubercidin. He is also willing to try to obtain analytically pure samples of 5'-deoxynebularine, 5'-chloro-5'-deoxytubercidin, and additional 5'-deoxytubercidin for screening from material now in hand. Probably these samples only require recrystallization.

## EXPERIMENTAL SECTION

Melting points were obtained on a Mel-Temp block and are uncorrected. In most cases melting points were variable and prior softening was encountered even with analytically pure samples. Therefore, UV and tlc were used as prime criteria of purity. Tlc was performed on Eastman 13254 cellulose with fluorescent indicator in Systems: A (1-butanol-H<sub>2</sub>O, 7:2:1, v/v), and C (1-butanol-EtOH-H<sub>2</sub>O, 10:3:7:, v/v). R<sub>f</sub> values of the compounds are contained in Table I. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. 6-chloropurine riboside was purchased from Sigma. Tubercidin was purchased mainly from Sigma but, when this supplier was out of stock, from Vega Biochemicals or P-L Biochemicals.

5'-chloro-5'-deoxytubercidin<sup>8</sup> (2). - The general method of Kikugawa and Ichino<sup>9</sup> was followed with modification only in the isolation procedure. To a stirred, chilled solution of dry 1 (5.0g, 18.8 mmole) in 50 ml of dry hexamethylphosphoramide was added 7.5 ml of freshly distilled SOCl<sub>2</sub>. After 18 hours at 70° the red mixture was poured into 450 ml of ice-cold H<sub>2</sub>O. The suspension was chromatographed over Dowex 50w-x4 [H+] resin by elution with H<sub>2</sub>O until the eluate was no longer acidic, and then with a linear gradient of 0.1-2N NH<sub>4</sub>OH. The fractions which had max at 270nm were pooled. Evaporation of solvent in vacuo below 35° followed by drying under high vacuum at room temperature gave 5.34g (100%) of amorphous white powder, homogeneous on tlc in systems A, B, and C.  $\lambda_{\text{max}}^{0.1N \text{ NH}_4\text{OH}}$  270 nm,  $\epsilon=610 \text{ cm}^{-1}$  (C-Cl stretch). mp 140-42° (prior softening). NMR(DMSO-D<sub>6</sub>)  $\delta$  3.9 (m, 2H, C<sub>5</sub>'-H), 4.1 (m, 2H, C<sub>2</sub>'-H and C<sub>3</sub>'-H), 4.5 (m, 1H, C<sub>4</sub>'-H), 5.4 (br, 2H, OH), 6.0 (d, 1H, C<sub>1</sub>'-H), 6.9 (br, 2H, -NH<sub>2</sub>), 6.6, 7.3 (2s, 2H, C<sub>7</sub>-H and C<sub>8</sub>-H), 7.9 (s, 1H, C<sub>2</sub>-H).

A sample of 2 isolated during attempted synthesis of 5'-deoxy-5'-fluoro-tubercidin (see text) gave the following elemental analysis for C<sub>11</sub>H<sub>13</sub>ClN<sub>4</sub>O<sub>3</sub> (MW=284.70).

	PERCENT			
	C	H	N	Cl
Theory:	46.41	4.60	19.68	12.45
Found:	46.43	4.46	19.40	13.12

5'-Deoxytubercidin<sup>12</sup> (3)- Compound 3 was prepared by the general method of Wang and Hogenkamp.<sup>10</sup>

To a stirred solution of dry 2 (1.0g, 3.5mmole) in THF (freshly distilled from sodium and dried over molecular sieves) was added 4g (13.8 mmole) of tributyltin hydride (Alpha Inorganics) and 0.25g (1.5 mmole) of azobis (isobutyronitrile) (AIBN, Aldrich). The mixture was refluxed under N<sub>2</sub> for 24 hr. The solvent was removed in vacuo and the residue solidified by addition of cold petroleum ether. The crude product was collected by filtration, washed with 100 ml cold petroleum ether, and recrystallized twice from reagent alcohol to give 0.31g (30%) of white crystals, mp 182-84° (prior softening)  $\lambda_{\text{max}}^{0.1N\text{NaOH}}$  = 270 nm ( $\epsilon_{\text{max}}$  - 1.2x10<sup>4</sup>). Homogeneous in the systems A,B, and C. IR and NMR spectra were compatible with the structure\*. Anal. For C<sub>11</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub> (MW=250.26):

	% C	% H	% N
Calcd:	52.79	5.64	22.39
Found:	52.39	5.68	22.09

5'-Deoxy-2',3'-O-isopropylidenetubercidin (5). - The general procedure of Wechter and Hanze<sup>19</sup> was employed.

To a stirred solution of 10mg of 3 in 1.0ml of dry acetone and 0.2ml of 2,2-dimethoxypropane was added 30 mg of p-toluenesulfonic acid. After 2 hrs. tlc showed complete disappearance of 3 and formation of a new product (5) which was homogeneous and moved with the solvent front (Table 1).

5'-Deoxy-5'-iodonebularine (7). - A solution of 14.9g (33 mmole) of methyltriphenoxyposphonium iodide<sup>20</sup> in DMF (83ml, total volume) was added via an additional funnel to a 200ml chilled flask containing 5.6g (22 mmole) of dry nebularine.<sup>18</sup> The reaction mixture was stirred for 6 hrs in an ice-bath. More Rydon reagent<sup>20</sup> (4.5g in 25ml of DMF) was then added. After 3 hrs, the mixture was poured into 300 ml of 10% aqueous sodium thiosulfate, extracted with ethyl acetate (9x200ml). Organic layers were pooled and concd in vacuo to give a yellow oil, which was dissolved in 25ml of DMF and chromatographed on 32 sheets of Whatman 3MM paper in System C. The fastest band was phenol. Strips containing the product band, which moved between phenol and unreacted nebularine, were eluted by descending chromatography in 2-propanol-H<sub>2</sub>O (7:3, v/v). The eluate was collected in beakers, pooled, and concd in vacuo to give a yellow oil, which was dissolved in 100ml of CHCl<sub>3</sub>-MeOH (3:1, v/v) and applied to a column of 300g of silica gel prepared with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (15:1, v/v) and eluted with the same solvent system. Fractions 3-16 (100 ml ea) were found by uv to contain product, were pooled and evaporated to give 0.96g of a tan powder which could not be recrystallized from any of several solvents but was chromatographically homogeneous and had the same uv spectrum as nebularine ( $\lambda_{\text{max}}^{0.01N\text{NaOH}}$ =262nm). A small sample of 7 was found by tlc to decompose completely after 6 hr in boiling H<sub>2</sub>O to give nebularine and a product presumed to be N<sup>3</sup>,5'-cyclonebularine (Rf=0.41 in system A). In this property compound 7 thus resembles other 5'-deoxy-5'-iodonucleosides<sup>12,20</sup>. Compound 7 was used without further purification for the next step.

\*The NMR spectrum resembled that of other 5'-deoxynucleosides. See ref. 10.

5'-Deoxynebularine 8. - To a stirred solution of 1.2g (3.4 mmoles) of crude 7 in 40ml of ethanol was added 600mg of 5% Pd on charcoal and 3.0g of sodium acetate. Hydrogenation was conducted overnight at ambient temperature and pressure. When tlc showed the reaction was complete, the catalyst was removed by filtration and washed thoroughly with hot ethanol. The filtrate was evaporated to dryness to give a yellow solid which was taken up in 50ml of CHCl<sub>3</sub>-MeOH (1:1 v/v) and applied to a column of 200g of silica gel, prepared in and eluted with CH<sub>2</sub>Cl<sub>2</sub>-EtOH (15:1, v/v). Twenty-eight fractions (200ml each) were collected. Fractions 16-26 were found by uv to contain the product and were pooled and evaporated to give a white-gray solid. Attempted recrystallization from 1-propanol gave only sodium acetate. Mother liquors containing 8 were evaporated to give a brown residue, which awaits further purification. A small sample of 8 was quantitatively converted to isopropylidene derivative 9 as confirmation of structure. (Table I). Table II contains a list of samples submitted to Walter Reed Army Institute of Research during the course of the project.

TABLE I

Rf Values of Tubercidin and Nebularine Derivatives

<u>Compound No.</u>	<u>SYSTEM</u>		
	<u>A</u>	<u>B</u>	<u>C</u>
<u>1</u>	0.49	0.73	0.67
<u>2</u>	0.77	0.84	0.78
<u>3</u>	0.72	0.81	0.74
<u>4</u>	0.99	0.99	0.99
<u>5</u>	0.99	0.99	0.99
<u>6</u>	0.50	0.75	0.67
<u>7</u>	0.88	0.90	0.87
<u>8</u>	0.85	0.92	0.81
<u>9</u>	0.99	0.99	0.99

TABLE II

Compounds Submitted for Antiparasite Screening During Contract Period\*\*

<u>Compound</u>	<u>Sample No.</u>	<u>WR Bottle No.</u>	<u>Amount</u>
Nebularine	PL-I-12	BJo8205	1.6g
5'-Deoxytubercidin	SA-32	BJ30583	343mg
5'-Deoxytubercidin	SA-41	BJ30592	257mg

\*\*Submitter key no. 1269. Samples of 5'-deoxynebularine, 5'-chloro-5'-deoxytubercidin, and additional 5'-deoxytubercidin will be submitted if the principal investigator can render them analytically pure by recrystallization of material now in hand.

# LITERATURE CITED

1. B.R. Baker, J.P. Joseph, and J.H. Williams J.Amer.Chem.Soc., 77, 1 (1955).
2. J.J. Jaffe, Ann.N.Y.Acad.Sci., 255, 306 (1975).
3. J. Williamson and T.J. Scott-Finnigan, Antimicrob.AgentsChemother., 13, 735 (1978).
4. K.E. Kinnamon, E.A.Steck, and D.S. Rane, ibid., 15, 157 (1979).
5. J.P. Neenan and W. Rohde, J.Med.Chem., 16, 580 (1973).
6. T-S. Lin, J.P. Neenan, Y-C. Cheng, W.H. Prusoff, and D.C. Ward, ibid., 19, 495 (1976)
7. P. Langen and G. Kowollik, Eur.J.Biochem., 6, 344 (1968).
8. R.T. Borchardt, J.A. Huber, and Y-S. Wu, J.Med.Chem., 19, 1094 (1976)
9. K. Kikugawa and M. Ichino, Tetrahedron Lett., 87 (1971).
10. Y. Wang and H.P.C. Hogenkamp, Carbohydr.Res., 59, 449 (1977).
11. W. Jahn, Ber., 98, 1705 (1965).
12. K. Anzai and M. Matsui, Bull.Chem.Soc.Jap., 46, 618 (1973).
13. C.W. Tullock and D.D. Coffman, J.Org.Chem., 25, 2016 (1960).
14. See the collected volumes of "Reagents for Organic Synthesis," M. Fieser and L.F. Fieser, Wiley, N.Y. for leading references.
15. A. Hampton, M. Bayer, U.S. Gupta, and S.-Y. Chu, J.Med.Chem., 11, 1229 (1968).
16. T. Hata., I. Yamamoto, and M. Sekine, Chem.Lett., 977 (1975).
17. K.A. Watanabe and J.J. Fox, Agnew.Chem.Int.Ed.Eng. 5, 579 (1966).
18. G.B. Brown and V.S. Weliky, J.Biol.Chem., 204, 1019 (1953).
19. W.J. Wechter and A.R. Hanze, U.S. Patent 3,336,289. to Upjohn Co. (1967).
20. J.P.H. Verheyden and J.G. Moffatt, J.Org.Chem., 35, 2319 (1970).  
S.D. Dimitrijevic, J.P.H. Verheyden, and J.G. Moffatt, J.Org.Chem., 44, 400 (1979).

12 Copies

Director (ATTN: SGRD-UWZ-AG)  
Walter Reed Army Institute of  
Research  
Walter Reed Army Medical Center  
Washington, D. C. 20012

4 Copies

HQDA (SGRD-SI)  
Fort Detrick  
Frederick, MD 21701

12 Copies

Defense Technical Information Center (DTIC)  
ATTN: DTIC-DDA  
Cameron Station  
Alexandria, Virginia 22314

1 Copy

Dean  
School of Medicine  
Uniformed Services University  
of the Health Sciences  
4301 Jones Bridge Road  
Bethesda, Maryland 20014

1 Copy

Superintendent  
Academy of Health Sciences, US Army  
ATTN: AHS-COM  
Fort Sam Houston, Texas 78234



REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER (9) Annual rept. no. 1 (Final)	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) (6) SYNTHESIS OF PURINE NUCLEOSIDE AND NUCLEOTIDE ANALOGS AS ANTIPARASITIC AGENTS		5. TYPE OF REPORT & PERIOD COVERED Annual and Final 1 September 1978 - 31 August 1979
7. AUTHOR(s) (10) John P. Neenan / Ph.D.		8. CONTRACT OR GRANT NUMBER(s) (15) DAMD17-78-C-8058
9. PERFORMING ORGANIZATION NAME AND ADDRESS Illinois Benedictine College 5700 College Rd. Lisle, IL 60532 (14) 4		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (16) 62770A-BM162770A803 00/050
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		12. REPORT DATE (11) 1 September 1979
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) (12) 17		13. NUMBER OF PAGES 16
		15. SECURITY CLASS. (of this report) Unclassified
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES 79 12 31 015		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Synthesis of purine nucleoside and nucleotide analogs as antiparasitic agents.		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) In this research studies on the synthesis of purine nucleoside and nucleotide analogs as antiparasitic agents were conducted. Primary target compounds were 5'-deoxytubercidin 5'-amino-5'-deoxytubercidin and 5'-fluoro-5'-deoxytubercidin. One target, 5'-deoxytubercidin was successfully prepared in an efficient two-step procedure and submitted for screening. Attempts to prepare the 5'-amino- and 5'-fluoro-derivatives of tubercidin by direct routes or via blocked tubercidin were unsuccessful. Work on 5'-derivatives of nebularine was performed and some success was achieved in this series. A two-step synthesis of 5'-deoxynebularine from nebularine via		

Block 20 - (continued)

5'-deoxy-5'-iodonebularine was accomplished.

Additional 5'-deoxytubercidin and its synthetic precursor, 5'-chloro-5'-deoxytubercidin were prepared. These samples are not analytically pure as of this report.

In summation, 1.6g of nebularine and over 600 mg of 5'-deoxytubercidin were submitted for screening during the project. Additional 5'-deoxytubercidin as well as samples of 5'-chloro-5'-deoxytubercidin and 5'-deoxynebularine are in hand but require further purification.

## INSTRUCTIONS FOR PREPARATION OF REPORT DOCUMENTATION PAGE

**RESPONSIBILITY.** The controlling DoD office will be responsible for completion of the Report Documentation Page, DD Form 1473, in all technical reports prepared by or for DoD organizations.

**CLASSIFICATION.** Since this Report Documentation Page, DD Form 1473, is used in preparing announcements, bibliographies, and data banks, it should be unclassified if possible. If a classification is required, identify the classified items on the page by the appropriate symbol.

### COMPLETION GUIDE

**General.** Make Blocks 1, 4, 5, 6, 7, 11, 13, 15, and 16 agree with the corresponding information on the report cover. Leave Blocks 2 and 3 blank.

**Block 1.** Report Number. Enter the unique alphanumeric report number shown on the cover.

**Block 2.** Government Accession No. Leave Blank. This space is for use by the Defense Documentation Center.

**Block 3.** Recipient's Catalog Number. Leave blank. This space is for the use of the report recipient to assist in future retrieval of the document.

**Block 4.** Title and Subtitle. Enter the title in all capital letters exactly as it appears on the publication. Titles should be unclassified whenever possible. Write out the English equivalent for Greek letters and mathematical symbols in the title (see "Abstracting Scientific and Technical Reports of Defense-sponsored RDT/E," AD-667 000). If the report has a subtitle, this subtitle should follow the main title, be separated by a comma or semicolon if appropriate, and be initially capitalized. If a publication has a title in a foreign language, translate the title into English and follow the English translation with the title in the original language. Make every effort to simplify the title before publication.

**Block 5.** Type of Report and Period Covered. Indicate here whether report is interim, final, etc., and, if applicable, inclusive dates of period covered, such as the life of a contract covered in a final contractor report.

**Block 6.** Performing Organization Report Number. Only numbers other than the official report number shown in Block 1, such as series numbers for in-house reports or a contractor/grantee number assigned by him, will be placed in this space. If no such numbers are used, leave this space blank.

**Block 7.** Author(s). Include corresponding information from the report cover. Give the name(s) of the author(s) in conventional order (for example, John R. Doe or, if author prefers, J. Robert Doe). In addition, list the affiliation of an author if it differs from that of the performing organization.

**Block 8.** Contract or Grant Number(s). For a contractor or grantee report, enter the complete contract or grant number(s) under which the work reported was accomplished. Leave blank in in-house reports.

**Block 9.** Performing Organization Name and Address. For in-house reports enter the name and address, including office symbol, of the performing activity. For contractor or grantee reports enter the name and address of the contractor or grantee who prepared the report and identify the appropriate corporate division, school, laboratory, etc., of the author. List city, state, and ZIP Code.

**Block 10.** Program Element, Project, Task Area, and Work Unit Numbers. Enter here the number code from the applicable Department of Defense form, such as the DD Form 1498, "Research and Technology Work Unit Summary" or the DD Form 1634, "Research and Development Planning Summary," which identifies the program element, project, task area, and work unit or equivalent under which the work was authorized.

**Block 11.** Controlling Office Name and Address. Enter the full, official name and address, including office symbol, of the controlling office. (Equates to funding/sponsoring agency. For definition see DoD Directive 5200.20, "Distribution Statements on Technical Documents.")

**Block 12.** Report Date. Enter here the day, month, and year or month and year as shown on the cover.

**Block 13.** Number of Pages. Enter the total number of pages.

**Block 14.** Monitoring Agency Name and Address (if different from Controlling Office). For use when the controlling or funding office does not directly administer a project, contract, or grant, but delegates the administrative responsibility to another organization.

**Blocks 15 & 15a.** Security Classification of the Report: Declassification/Downgrading Schedule of the Report. Enter in 15 the highest classification of the report. If appropriate, enter in 15a the declassification/downgrading schedule of the report, using the abbreviations for declassification/downgrading schedules listed in paragraph 4-207 of DoD 5200.1-R.

**Block 16.** Distribution Statement of the Report. Insert here the applicable distribution statement of the report from DoD Directive 5200.20, "Distribution Statements on Technical Documents."

**Block 17.** Distribution Statement (of the abstract entered in Block 20, if different from the distribution statement of the report). Insert here the applicable distribution statement of the abstract from DoD Directive 5200.20, "Distribution Statements on Technical Documents."

**Block 18.** Supplementary Notes. Enter information not included elsewhere but useful, such as: Prepared in cooperation with . . . Translation of (or by) . . . Presented at conference of . . . To be published in . . .

**Block 19.** Key Words. Select terms or short phrases that identify the principal subjects covered in the report, and are sufficiently specific and precise to be used as index entries for cataloging, conforming to standard terminology. The DoD "Thesaurus of Engineering and Scientific Terms" (TEST), AD-672 000, can be helpful.

**Block 20.** Abstract. The abstract should be a brief (not to exceed 200 words) factual summary of the most significant information contained in the report. If possible, the abstract of a classified report should be unclassified and the abstract to an unclassified report should consist of publicly-releasable information. If the report contains a significant bibliography or literature survey, mention it here. For information on preparing abstracts see "Abstracting Scientific and Technical Reports of Defense-Sponsored RDT&E," AD-667 000.